

**Project Title: “Demography, Community Dynamics, and Health of Reintroduced Wood Frog Populations and Resident Amphibian Communities in Restored Ephemeral Wetlands and Oak Woodlands in Lake County, IL” - T-107-D-1**

Submitted by:

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**Background:**

In the Upper Des Plaines River Conservation Opportunity Area, decades of habitat degradation have dramatically affected the region’s historic assemblage of amphibians. Recently, the Lake County Forest Preserve District (LCFPD) embarked on a major habitat restoration initiative in MacArthur Woods Forest Preserve, followed by the reintroduction of several priority amphibian species that were unlikely to recolonize naturally. The activities proposed here will allow the Primary Investigators (PIs) to effectively evaluate the success of these efforts, and collect additional data needed to inform future amphibian conservation actions in Illinois.

First, this project will provide long-term post-translocation monitoring data for the amphibian species reintroduced to MacArthur Woods: **wood frogs (*Lithobates sylvaticus*)**, an Illinois Species in Greatest Need of Conservation, **spotted salamanders (*Ambystoma maculatum*)**, a regionally rare species in the northeastern morainal division of Illinois, and **spring peepers (*Pseudacris crucifer*)**, a species that may be undergoing local decline in northeastern Illinois (Mierzwa 1999, Dan Thompson- Forest Preserve District of DuPage County Pers. Comm. 2014, Bill Graser- Forest Preserve District of Kane County, Pers. Comm., 2015). Second, a demographic study of reintroduced amphibians and resident amphibians will facilitate the use of population projections to determine if additional conservation actions are necessary for persistence. Third, this project will examine amphibian response to LCFPD’s habitat restoration efforts in MacArthur Woods and neighboring Des Plaines River sites, with regard to changes in community structure, diversity, and abundance. Finally, this project will provide a much-needed examination of amphibian stress and disease dynamics as habitat suitability changes in a fragmented, urbanized landscape.

*History of the MacArthur Woods Amphibian Community*

MacArthur Woods Forest Preserve is a Lake County Forest Preserve and Illinois Nature Preserve in the Upper Des Plaines River Conservation Opportunity Area. Baseline monitoring during the 1980s and 1990s documented the decline and eventual extirpation of three key amphibian species from the site: wood frogs (an Illinois Species in Greatest Need of Conservation), spotted salamanders, and spring peepers. Between 1983 and 1999, severe levels of infestation by European buckthorn, altered hydrology from the installation of agricultural drainage tile, and a regional history of fire suppression contributed to the loss of these species from the preserve (Mierzwa 2001). In the late 1990s, only three amphibian species persisted in MacArthur Woods: blue-spotted salamanders (*Ambystoma laterale*, another Illinois Species in Greatest Need of Conservation), western chorus frogs (*Pseudacris triseriata*), and American toads (*Anaxyrus americanus*). Blue-spotted salamanders represented approximately 85% of the site’s amphibian community (Mierzwa 2001).

In 1999, the LCFPD began an intensive habitat restoration initiative targeting flatwoods wetlands in MacArthur Woods. Major restoration efforts included disablement of 916 lineal meters of agricultural drainage tile which rehydrated 100 acres of ephemeral wetlands, improving breeding pond hydroperiods for amphibians (Klick 2003), and the removal of 300+ acres of European buckthorn. Buckthorn impedes hatching success for resident amphibians because it releases the compound emodin, which disrupts amphibian embryo development (Sacerdote and King 2014). Other restoration management actions included replanting of native shrubs and trees, and prescribed burns to encourage regeneration of native ground cover (Klick 2003). Intensive habitat restoration continued through 2003 and management, including European buckthorn control, is ongoing.

As a result of the LCFPD's restoration efforts in MacArthur woods, several amphibian species naturally colonized the site. These included tiger salamanders (*Ambystoma tigrinum*), green frogs (*Lithobates clamitans*), northern leopard frogs (*Lithobates pipiens*), and bullfrogs (*Lithobates catesbeianus*). Unfortunately, LCFPD officials determined that natural recolonization of the extirpated amphibian species (wood frogs, spotted salamanders, and spring peepers) was unlikely due to lack of source populations in nearby preserves. In conjunction with LCFPD, the PI and Northern Illinois University researchers conducted a reintroduction feasibility assessment examining aquatic survival for these species between 2005-2006 (Sacerdote 2009, Sacerdote & King, 2009), and implemented translocations between 2007 and 2010. The PI placed three cohorts of wood frog eggs, three cohorts of spotted salamander eggs, and two cohorts of spring peeper larvae into several replicate *in situ* pond enclosures in five seasonal ponds in MacArthur Woods. She monitored hatching success for translocated wood frogs and spotted salamanders, as well as resident blue-spotted salamanders. She also monitored larval survival to metamorphosis for wood frogs, spotted salamanders, spring peepers, and blue-spotted salamanders (Sacerdote 2009).

The reintroductions produced a total of 55, 213, and 146 spotted salamander metamorphs for release in 2007, 2008, and 2009, respectively. In 2008, only two wood frog egg clutches were translocated due to timing of egg collection, with a total of 87 metamorphs released. In the 2009 and 2010 translocations of wood frog egg masses, however, more than 5,000 tadpoles hatched in MacArthur Woods. The PI maintained a subset of 150 tadpoles in enclosures to measure larval survival rates, and released the remaining tadpoles into the ponds. Of the enclosed tadpoles, 99 and 126 wood frogs survived to metamorphosis in the enclosures in 2009, and 2010, respectively. Finally, she released 50 spring peeper metamorphs both in 2008 and 2009.

Follow-up monitoring for reintroduced amphibians has occurred every year since translocation began. Monitoring activities include anuran call monitoring, egg mass surveys, dip-netting, and cover searches during breeding migration to ponds and post-metamorphic emigration from ponds. However, because of resource constraints, drift fences and larval sampling have not been employed throughout each season, and individuals have not been marked for long-term identification, which would permit estimation of population size. This proposal will increase survey efforts, including the incorporation of mark-recapture-release to examine the changes in community structure and demography, as additional oak woodland restoration management takes place in MacArthur Woods and in neighboring preserves along the Des Plaines River corridor.

Thus far, post-reintroduction monitoring has documented important preliminary outcomes. Since reintroduction and translocation efforts were implemented, spring peepers were consistently documented calling in two study ponds, indicating survival to reproductive stage. As of 2014, wood frogs

were also documented persisting in the site, with breeding adults in two study ponds, and approximately 50 egg masses in each of two ponds. In 2014, egg masses were monitored for hatching success, and successful metamorphs were observed around the breeding ponds. Despite these successes for two focal species, monitoring teams have not yet observed spotted salamanders breeding in MacArthur Woods. However, juvenile spotted salamanders spend ~90% of their time below ground in small mammal burrows (Regosin, Windmiller, & Reed, 2003), so until they reach reproductive age (approximately five years) their detection probability is quite low. While wood frogs typically take 2-3 years to reach reproductive maturity (Berven, 2009), no reproduction was observed until four years after the most recent translocation in 2010. A series of consecutive drought years in 2012 and 2013 may have limited reproductive activity in these focal species.

Another factor which may influence establishment of self-sustaining populations of reintroduced wood frogs, spotted salamanders, and spring peepers into additional study ponds is the natural colonization of the site by tiger salamanders. Prior to restoration, tiger salamanders were not observed in MacArthur Woods. They were first detected at the site in a semi-permanent pond along the Des Plaines River floodplain in 2009 (Sacerdote 2009). Several adults have been observed at the floodplain pond and alongside the largest of the interior flatwoods ponds every year since 2009. In 2014, 13 tiger salamander egg masses were observed in three of the five main breeding ponds in the site. Tiger salamanders are the dominant vertebrate predators in the ephemeral pond community. As such, they may outcompete or depredate eggs and larvae of other Ambystomatid salamanders, reducing the likelihood of establishment by spotted salamanders.

Changes in the MacArthur Woods amphibian community through applied conservation management as well as natural colonization necessitate the examination of how the resident and reintroduced species respond to changes in community structure. This need will increase as habitat suitability in the Upper Des Plaines River Corridor Conservation Opportunity Area continues to improve due to ongoing restoration efforts, facilitating interpond and intersite movement for amphibians. As more amphibian species are able to colonize restored ponds, reintroduced species may establish in additional ponds or in neighboring sites. However, improved migration may also increase the movement of amphibian diseases such as chytridiomycosis (“chytrid”) on the landscape.

#### *Amphibian Disease and Stress*

The spread of the waterborne fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), the causative agent of chytrid, has been linked to the extinction of almost 200 amphibian species in the last two decades alone (Vredenburg, Knapp, Tunstall, & Briggs, 2010). *Bd* is a unique fungal skin pathogen that infects keratinized tissues preventing enough water, oxygen and other important ions from getting across their skin (Rollins-Smith, Ramsey, Pask, Reinert, & Woodhams, 2011). The pathogen remains confined to the site of infection and in larvae, impacts of the infection often are sub-lethal so infected individuals can maintain the pathogen in the environment after adults have dispersed (Briggs, Knapp, & Vredenburg, 2010; Rollins-Smith et al., 2011). Newly metamorphic frogs are especially vulnerable because their immune systems are still developing (Rollins-Smith et al., 2011). Infection in post-metamorphic amphibians is typically fatal (Briggs et al., 2010). However, some species of adult amphibians may harbor and vector *Bd* zoospores but not succumb to chytrid. Because of this, infected but asymptomatic adult amphibians may vector the disease to new locations, and may be subject to reinfection if infected larvae are present in the environment (Briggs et al., 2010; Talley & Lipps, 2010). Zoospore load and resistance also varies across amphibian species possibly impacting transmission dynamics (Briggs et al., 2010; Kilpatrick, Briggs, & Daszak, 2010). Therefore, assessments of the presence

and transmission dynamics of the *Bd* pathogen in the environment are of increasing global importance, particularly as they relate to proposed conservation activities like translocation of animals.

Amphibians are also impacted by a plethora of stressors, including habitat alteration and degradation, fragmentation, isolation, poaching, and contamination, and higher stress can cause greater susceptibility to disease (Padgett & Glaser, 2003). The link between health and stress may be especially significant in amphibians affected by *Bd* because as the pathogen attacks amphibians' immune defenses of the skin (Rollins-Smith et al., 2011). Additionally, the complex amphibian life cycle – including metamorphosis and intensive breeding – could result in greater susceptibility to *Bd* infections because of naturally elevated glucocorticosteroid hormones and immune suppression (Rollins-Smith et al., 2011). The assessments of amphibian health and stress proposed here aim to increase our understanding of the relationship between *Bd* susceptibility and environmental stressors, which could inform wildlife management strategies for controlling the disease.

Over the years, scientists have used blood and urine to measure stress hormones in amphibians (Narayan & Hero, 2011; Narayan, 2013). However, using blood is very invasive and using urine can be unreliable, as animals do not always urinate when sampled. Along with Lincoln Park Zoo colleagues, the PI has developed an innovative, reliable, and noninvasive method for measuring amphibian stress hormones that can be collected in the field during routine swabbing for *Bd*, further leveraging field sampling efforts and reducing potential negative impacts on the animals.

## **1. Need**

The Illinois Wildlife Action Plan calls for reintroduction of native species into habitat where decimating factors have been eliminated and natural recovery is unlikely. While the reintroduction of these priority species is a central goal of both the Action Plan's Wetlands and the Forest and Woodlands Campaigns, few amphibian translocation efforts have incorporated the intensive follow-up monitoring necessary to evaluate the success of these projects (Muths and Dreitz 2008). As part of the Southern Des Plaines Restoration Project, the LCFPD is conducting oak woodland and wetlands restoration in MacArthur Woods Forest Preserve and neighboring sites. These areas are key parcels of preserved land within the Upper Des Plaines River Conservation Opportunity Area. After years of intensive restoration efforts, wood frog and spotted salamander eggs and spring peeper larvae were translocated to MacArthur Woods when the extirpated species failed to recolonize naturally. Despite the initial survival of over 300 spotted salamander metamorphs, no adults have been documented at the site. In contrast, wood frogs, an Illinois Species in Greatest Need of Conservation, were recorded breeding in MacArthur Woods in 2014, with at least 50 egg masses and successful recruitment in each of two ponds. Similarly, spring peepers, with a short generation time, persisted and were breeding in two ponds following translocation. Further monitoring is necessary to assess if self-sustaining populations will establish and colonize additional ponds and preserves. With the devastating fungal pathogen chytrid now present in Illinois, there is also an immediate need to examine regional amphibian health and stress, as restoration improves site connectivity and influences local epidemiology. This project will monitor success of reintroduced wood frogs, spotted salamanders, and spring peepers, and examine the demography, community dynamics, health, and stress of both reintroduced and resident amphibian populations in MacArthur Woods in response to the LCFPD's restoration work. These critical data will be used to inform future restoration and translocation efforts for priority amphibian species in Illinois and beyond.

While presence of *Bd*, the causative agent of chytrid, has been documented in several central and southern Illinois watersheds (*Bd-maps.net*, Mulleman and Montgomery 2013, Phillips *et al.* 2014), the natural areas within Chicago and the surrounding counties (referred to as the Chicago Wilderness Region) had not been examined prior to 2014. Within the Chicago Wilderness Region, land use around fragmented natural areas strongly varies from urban to suburban to rural as one moves north, west, and south of Chicago. As habitat restoration efforts progress within this area, there is great interest in wildlife restoration and translocation to support regional populations of amphibians when fragmentation impedes natural colonization. However, the presence and distribution of *Bd* in the region is poorly understood, and there is a pronounced need for surveillance to improve knowledge regarding threats to the extant populations of amphibians prior to considering any translocation efforts. The need for surveillance of *Bd* in the region is especially critical for wood frogs, which are particularly susceptible to *Bd* (Gahl, Pauli, & Houlahan, 2011). A disease outbreak would thwart this priority species' successful establishment following reintroduction.

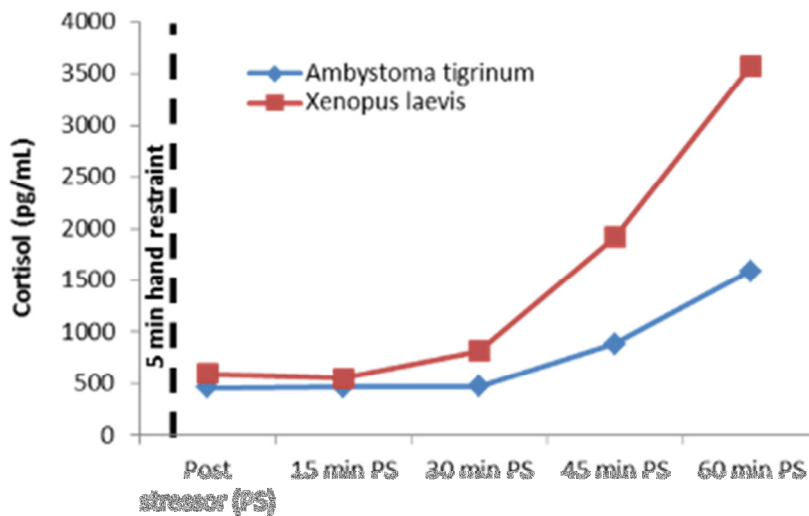
During the summer of 2014, Lincoln Park Zoo PIs conducted an initial *Bd* survey in the Chicago Wilderness Region of northeast Illinois. This was the first *Bd* survey of the region and, unfortunately, the team detected *Bd*. In July and August 2014, PIs collected 104 *Bd* swabs from lentic habitats in 20 sites across six counties (Cook, Lake, DuPage, Kane, McHenry, Boone). For this initial survey effort, they focused on sampling species that are often reservoirs for *Bd*, including American bullfrogs, green frogs, and northern leopard frogs. Swabs were also opportunistically collected from additional species. All survey sites were within forest preserves or parks where habitat restoration management efforts have been implemented, including invasive shrub clearing, prescribed burning, disablement of agricultural drainage tile, and conversion of row crop into grassland.

Six of the 20 (30%) study sites produced positive *Bd* samples, including sites in Cook, McHenry, DuPage, and Kane counties. The positive sites were located within six watersheds. Twelve of 104 (11.5%) amphibians sampled tested positive for presence of *Bd* zoospores, and *Bd* was detected in 20% of bullfrogs, 13% of northern leopard frogs, and 5% of green frogs sampled. All positive animals were either adults or metamorphs; no tadpoles tested positive for *Bd*.

The project's PIs have already disseminated their findings to wildlife officials in the Chicago Wilderness Region, and recommend changes in management based on the documented presence of *Bd*. As a result, Kane and DuPage counties have implemented new decontamination protocols for land management staff and contractors, and are collaborating with the PIs on additional surveillance efforts in their counties. However, further study is needed to more fully understand how this disease is currently impacting the region's amphibian populations, and how conservation efforts – particularly translocation efforts – may affect disease transmission dynamics.

During their initial *Bd* surveys, the PIs also tested new methodology to noninvasively measure amphibian stress levels using dorsal swabs. They collected 26 swabs to test for the stress hormone cortisol. Results from the stress swabs demonstrated that this methodology successfully detects cortisol. Preliminary results suggest that, in addition to expected interspecific differences in stress levels, there are also site differences in stress levels that may be attributed to environmental stressors as well as *Bd* infection status. The PIs worked with Lincoln Park Zoo endocrinologists to validate that this methodology is indeed measuring a physiological stress response. Using a captive-housed tiger salamanders (*Ambystoma tigrinum*) and an African clawed frog (*Xenopus laevis*), a mild acute stressor of

five minutes of hand restraint was induced. Baseline swabs were collected prior to hand restraint, immediately following hand restraint, and at 15 minute intervals for an hour. The swabs and extraction were successful in documenting the increase in cortisol in response to the stressor, which was expressed 45 minutes after restraint. This time lag indicates that field swabs will accurately represent a measure of stress based on amphibian response to the environment or disease, rather than capture or handling-related stress (Fig. 1).



**Figure 1.** Validation of detection of cortisol changes in response to a mild acute stressor (5 min hand restraint) in the tiger salamander (*Ambystoma tigrinum*) and the African Clawed Frog (*Xenopus laevis*) (From Manjerovic, Sacerdote-Velat, and Santymire, *In Prep.*).

## **2. Purpose & Objectives**

The purpose of this study is to collect demographic data for reintroduced and resident amphibian species in MacArthur Wood Forest Preserve. These data will be used to measure the efficacy of reintroduction following habitat restoration as a conservation tool for amphibians, and to examine the changes in amphibian community dynamics following both reintroduction and natural colonization. Additionally, the PIs propose to collect disease and stress data for reintroduced and resident amphibians in MacArthur Woods and surrounding preserves undergoing habitat restoration to more fully assess the impacts of these restoration efforts on regional amphibian populations.

Objectives:

(1) Collect demographic information for the reintroduced wood frog and spring peeper populations in MacArthur Woods, monitor for evidence of survival and recruitment for translocated spotted salamanders in MacArthur Woods, and examine potential colonization of reintroduced wood frogs and spring peepers into neighboring LCFPD sites in the Des Plaines River Corridor as Southern Des Plaines Restoration efforts progress. This objective will be implemented in Spring 2016 with the installation of drift fences. Surveys will commence as amphibians emerge and will continue through September

following the Pollock Robust Design identified in the Approach section. Surveys would span a 7 month period with 10 sampling days per month in each of six study sites. The survey efforts would be repeated in the same time window in 2017 and 2018. The technician will collect demographic data as captures permit, in each of the identified study sites. Data will be compiled by the PI's at the end of each sampling season, and a consolidated estimate of population size and population projections for wood frogs, spring peepers, and any spotted salamanders encountered will be completed by the PIs by January 2019.

(2) Collect demographic information for the resident amphibian communities (blue-spotted salamanders, western chorus frogs, American toads, tiger salamanders, green frogs, northern leopard frogs, and bullfrogs) in MacArthur Woods and surrounding LCFPD preserves undergoing oak woodland and wetlands restoration. This objective will be initiated in Spring 2016, with the installation of drift fences as survey efforts as described in objective 1. The surveys for seven potential resident species will be coincident with surveys for reintroduced wood frogs, spring peepers, and spotted salamanders. Data will be compiled at the end of each sampling season, and population projections for each species will be completed by the PIs by January 2019.

(3) Assess health and stress, including *Bd* presence, in reintroduced and resident amphibian communities in MacArthur Woods and neighboring LCFPD sites in the Des Plaines River Corridor as habitat restoration efforts progress. This objective will be completed on an ongoing basis each season as captures permit, with a target sample of up to 680 amphibians per season (42 individuals per study pond, with six individuals swabbed per primary sampling period in each pond). Analysis of stress swabs and *Bd* swabs will be completed by the following January each season (January 2017, 2018, and 2019). Stress swabs will be analyzed by the Lincoln Park Zoo endocrinology lab, and *Bd* swabs will be sent to Pisces Molecular Laboratory for analysis.

### **3. Expected Results & Benefits**

LCFPD Southern Des Plaines Restoration Project efforts are expected to encourage oak regeneration, decrease woodland fragmentation, provide greater leaf litter cover for migrating priority amphibians, and remove invasive shrubs that impact amphibian breeding habitat. Surveillance of amphibian populations during and after habitat restoration will allow PIs to assess the efficacy of these efforts.

Collection of detailed demographic data for reintroduced wood frogs and continued monitoring for spotted salamanders for three breeding seasons (2016-2018) will allow LPZ PIs to examine population size, population growth rate, and likelihood of persistence and colonization for wood frogs (Species in Greatest Need of Conservation), spotted salamanders, a regionally rare species, and spring peepers. These data will directly inform regional recovery efforts. Specifically, projection models will be used to guide future applied conservation management in MacArthur Woods and other restored forested wetlands, such as population supplementation, translocations, or additional reintroduction efforts.

The PIs will also examine changes in LCFPD resident amphibian community demographics, including resident blue-spotted salamanders (Species in Greatest Need of Conservation), with and without the presence of reintroduced wood frogs, as restoration is implemented. Demographic estimates generated from this study will increase our understanding of how restoration prescriptions and community

interactions may affect establishment of self-sustaining amphibian populations. These data will help guide future amphibian reintroduction projects, informing managers if additional releases are necessary to maintain populations of reintroduced amphibians.

Chytrid sampling will improve knowledge of regional disease status, interspecific differences in susceptibility, and changes in disease presence and prevalence as site connectivity improves in restored habitats. Examination of amphibian stress via cortisol sampling will elucidate changes in amphibian stress response as habitat quality improves and amphibian community dynamics change. Variation in stress response may also be linked to pathogen susceptibility and/or zoospore load. Identification of *Bd* positive sites will affect decontamination protocols for gear used by regional land managers and contractors.

Finally, the LCFPD's ongoing oak woodland and ephemeral wetlands restorations efforts, as key components of the Southern Des Plaines Restoration Project, will focus on phased removal of large overstory competitors, removal of invasive plant species, understory thinning in flatwoods, mesic, and dry mesic woodlands, and planting of native shrubs and trees. The expected improvements in ground cover, canopy openness above amphibian breeding ponds, and increased leaf litter in upland areas should facilitate interpond and intersite migration by wood frogs and other forested wetland amphibians, as these species require sufficient leaf litter for movement in upland habitats and canopy gaps to promote in-pond photosynthesis to provide phytoplankton and periphyton for grazing tadpoles, and zooplankton for salamander larvae (Rittenhouse & Semlitsch, 2007; Sacerdote & King, 2009).

#### **4. Approach**

##### **Lincoln Park Zoo**

Objectives 1 and 2:

- Job 1a: Beginning in February 2016, technician and PIs will install 12 three-arm drift fence arrays with four pitfall traps each and six funnel traps each, in MacArthur Woods, Old School, Grainger Woods, Wright Woods/Half Day, Elm Rood Woods, and Ryerson Woods Preserve. Four drift fence arrays will be installed in MacArthur Woods, one in Old School, one in Grainger Woods, two in Wright Woods, and four in Ryerson Woods.
- Job 1b: Technician and PIs will use mark-recapture approaches to collect species-specific abundance data for reintroduced amphibians (wood frogs, spotted salamanders, and spring peepers), and resident amphibians (blue-spotted salamanders, tiger salamanders, American toads, northern leopard frogs, western chorus frogs, American bullfrogs, and green frogs) during the 2016, 2017, and 2018 breeding seasons. Individual marks will be administered using a Visible Implantable Alphanumeric Tag (Northwest Marine Technologies) so that recaptures may be easily identified (Osborn *et al.* 2011). Photographs of individual salamanders will also be collected for photo-recognition software, Wild-ID (Bolger, Morrison, Vance, Lee, & Farid, 2012).
- Job 1c. The PIs will use the robust design (Pollock 1982) to permit site-by-site estimation of survival and abundance within each season using  $k$  primary sampling periods ( $k=7$  months: late February/early March, late March/early April, late April/early May, late May/early June, late June/early July, late July/early August, late August/early September) and  $l$  secondary sampling



periods ( $I = 10$  days within each month) with 2 weeks between each primary sampling period. The 2 week gap between primary sampling periods allows for changes in gains and losses in the populations (birth, death, immigration, and emigration), while the secondary sampling period is essentially closed to such changes (Pollock 1982). The secondary sampling periods will permit estimation of conditional capture and recapture probabilities, as well as abundance. Pooled capture probabilities within each month (primary sampling period) will be used to estimate apparent survival (Muths and Dreitz 2008). The PIs will examine effects of environmental covariates of interest (e.g. water chemistry, temperature, hydroperiods, canopy cover, soil moisture, leaf litter cover/depth) on capture and recapture probabilities, and apparent survival. The PIs will also examine effects of species interactions and structure of the amphibian assemblage (e.g. species richness, diversity, presence of competing species, and presence of predatory species) on capture, recapture, and survival probabilities. The abundance, survival, and reproductive rate data collected during mark-release-recapture in each season will be incorporated into matrix projection models to examine growth rate and likelihood of persistence over time (Caswell 2001). Model outcomes may be used to guide future applied conservation management such as population supplementation or additional translocations.

- Job 2: The technician will place SongMeters (SM2) at each study pond to continuously record for calling activity of reintroduced wood frogs and spring peepers, and resident frog species (American toad, American bullfrog, western chorus frogs, northern leopard frogs, green frogs) from mid-February through August of each year. Calling indices may be used to estimate number of breeding adults of each anuran species present in each study site (Royle, 2004).
- Job 3: The technician will conduct egg mass surveys in all study ponds and monitor wood frog and any spotted salamander egg masses to record hatching success. Egg mass surveys will facilitate breeding output (or number of reproductive females of each species present in each study site). Egg masses/strands will be identified to species using the USGS key to Amphibian Eggs and Larvae of Minnesota, Wisconsin, and Iowa (Parmalee *et al.* 2002). Egg mass counts will then be extrapolated to estimate number of breeding females. This approach will be especially useful for caudate amphibians which will not be detected in call surveys.
- Job 4: Technician and PIs will use standardized larval sampling to estimate relative abundance of amphibians using collapsible minnow traps and time-constrained dip-net surveys in study ponds. 40 minnow traps will be placed around the shoreline and along oviposition sites such as logs and emergent vegetation in each study pond. Time-constrained dip-net surveys will provide supplemental larval sampling. The use of both approaches will allow determination of larval density and diversity (Muths and Dreitz 2008).
- Job 5: Technician will monitor water chemistry parameters and environmental conditions throughout the study period using a multi-meter, canopy cover photography/Program Hemiphot (to examine canopy openness, leaf area index, photosynthetically-active photon flux density), temperature loggers, and rain gauges.

### Objective 3:

- Job 1: Technician/PIs will swab adult and post-metamorphic amphibians from drift fence captures for the amphibian pathogen *Batrachochytrium dendrobatidis* (Bd). Up to 680 amphibians per season (42 individuals per study pond, with six individuals swabbed per primary sampling period in each pond) will be ventrally swabbed (25x per sample) with a sterile cotton

swab to collect any *Bd* zoospores. Swabs will be placed in individual cryovials with 1 ml of 70 % ethanol. Standard chytrid disinfection protocol will be used on all equipment between sites. Cryovials will be prepared with unique identifier labels and will be shipped to Pisces Molecular Laboratory for analysis.

- Job 2: Pisces Molecular Laboratory (Boulder, CO) will analyze chytrid swabs to detect and quantify *Bd* zoospores using qPCR.
- Job 3: Technician/PIs will collect dorsal swabs of 120 amphibians per season using sterile cotton swabs (3x per sample) for cortisol. Swabs will be placed in individual cryovials with 1 ml of 70 % ethanol. The LPZ Endocrinology Laboratory will analyze cortisol swabs using enzyme immunoassay (EIA) to detect differences in intraspecific stress across study ponds in relation to environmental covariates (e.g. water chemistry) and community assemblage (e.g. presence of predatory amphibians).

## **5. Useful Life**

N/A

## **6. Geographic Location**

The study sites are located in the northeastern morainal division of Illinois, in Lake County. The focal sites, MacArthur Woods, Old School. Grainger Woods Conservation Area, Wright Woods, Elm Road Woods, and Ryerson Conservation Area are located within the Upper Des Plaines Conservation Opportunity Area, stretching along the Des Plaines River from Libertyville south to Lincolnshire, IL (Fig. 2).

The reintroduction site, MacArthur Woods, is located near Libertyville and Vernon Hills, IL (Fig. 3). The site is bound by the Des Plaines River to the west, the Canadian National Railroad to the north, St. Mary's Road to the east, and IL Route 60 to the south.

The Des Plaines River runs through all of the study sites potentially serving as a corridor for amphibian movement. Similarly, the study sites are connected by the Des Plaines River Trail.



Figure 2. Map of the proposed study sites for amphibian community dynamics, wood frog demography, and effects of oak woodland restoration on health and stress of amphibians. From north to south, study sites along the Des Plaines River include Old School Forest Preserve, MacArthur Woods Forest Preserve, Grainger Woods Conservation Area, Elm Road Woods Forest Preserve, Wright Woods Forest Preserve, and Ryerson Woods Conservation Area. MacArthur Woods is the reintroduction site for wood frogs, spotted salamanders, and spring peepers. The Des Plaines River forms a corridor through the study sites and the Des Plaines River Trail is an additional source of connectivity among study sites.



Figure 3. Map of MacArthur Woods Forest Preserve, the reintroduction site for wood frogs, spotted salamanders, and spring peepers. White stars indicate the ponds where wood frog breeding has been observed and yellow stars indicate the ponds where spring peeper breeding has been observed. The Des Plaines River is the western boundary of the site, with Canadian National Railroad to the north, St. Mary's Road to the east, and Route 60 to the south. The Des Plaines River Trail runs through the southern and eastern edge of the site, connecting MacArthur Woods to the other study sites.

## **7. Project Personnel**

The following Lincoln Park Zoo and LCFPD personnel will be the primary staff implementing the project:

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## **8. Program Income**

N/A



## **9. Budget Narrative**

### Budget Justification:

#### *Federal Funds*

#### Salaries, Wages and Fringe Benefits:

One technician will be hired to conduct field work as outlined in the objectives, for each project year.

The technician will be paid an hourly wage of \$12.00 per hour in 2016, and \$13.00 per hour in 2017 and 2018. The intern will work a total of 980 hours each year with FICA benefits of 7.65%.

#### Travel:

The technician will be provided with mileage reimbursement for travel between sampling sites, estimated at 1148 miles for the season, with a \$0.56/mile reimbursement rate.

#### Materials and Supplies:

Drift fence materials will include aluminum window screen mesh and metal posts for ten arrays, 40 5-gallon buckets for pitfall traps, and aluminum window screen mesh for funnel trap construction. Other amphibian sampling materials include 200 minnow traps, two dip nets, waders, 10 automated song meters with SD memory cards and batteries, visible implantable alphanumeric tags and an injector for marking captures, gear disinfection supplies (e.g. bleach, scrub brushes), and swab kits for chytrid and stress sampling. Individual materials and supplies are broken down on a cost per item basis (Table 1).

Table 1. Breakdown of materials and supplies with cost per unit and quantity.

Item	Cost Per Unit	Quantity	Total Cost
<b>Drift Fence Material</b>	\$ 355	1	\$ 355
<b>Pitfalls</b>	\$ 8	60	\$ 459
<b>Funnels</b>	\$ 5	90	\$ 494
<b>Minnow Traps</b>	\$ 10	200	\$ 2,000
<b>Dip Nets</b>	\$ 167	2	\$ 334
<b>Waders (6)</b>	\$ 70	6	\$ 420
<b>Song Meter SM2</b>	\$ 680	10	\$ 6,800
<b>Batteries and SD cards</b>	\$ 121	3	\$ 363
<b>Visible Implantable Alphanumeric Tags</b>	\$ 1	1530	\$ 1,530
<b>Tag injector kit with 100 tags</b>	\$ 310	2	\$ 620
<b>Chytrid Swab Kits</b>	\$ 50	15	\$ 750
<b>Corticosterone Swab Kits</b>	\$ 50	15	\$ 750
<b>Latex/Nitrile Gloves</b>	\$ 66	6	\$ 396
<b>Disinfection Supplies</b>	\$ 20	3	\$ 60
<b>Total</b>			<b>\$ 15,331</b>

#### Contractual:

Other costs include analysis of chytrid swabs via qPCR by Pisces Molecular Lab at \$70 per batch sample of 8 swabs, and analysis of cortisol samples by Lincoln Park Zoo's endocrinology lab at \$7.00 per sample.

#### *Non-Federal Match*

#### Contractual Services:

The non-federal match will be provided by Southern Des Plaines Restoration Project, a Lake County funded initiative that aims to encourage regeneration of oak woodlands through creation of light gaps, removal of invasive shrubs, and understory thinning. Regionally, oak regeneration is an issue of concern due to the history of fire suppression and invasion of understory shrubs. This restoration project targets historic oak woodland sites along the Des Plaines River in southern Lake County, including MacArthur Woods, Grainger Woods, Wright/Elm Road/Half Day Woods Complex, and the Ryerson Woods/Cahokia Complex. The Southern Des Plaines Restoration Project is funded entirely by Lake County Forest Preserve District's internal budget. No federal funding has been allocated toward the restoration project. The non-federal match will be provided through contractual services for Phase II and III restoration as part of the Southern Des Plaines Restoration Project, focused on clearing of invasive plants, understory removal, creation of light gaps, and selective canopy thinning to encourage oak and ground cover regeneration. These restoration actions are expected to benefit pond-breeding amphibians, including wood frogs, blue-spotted salamanders, and spotted salamanders by facilitating inter-wetland movement and improving pond hydroperiod through removal of invasive shrubs. Phase I of the Southern Des Plaines Restoration Project flatwoods restoration and oak woodland efforts in MacArthur Woods began in 2013. Phases II and III will be implemented during the proposed grant period, between 2016 and 2018. The restoration work that will provide matches for the requested federal funds includes major understory removal and light thinning in Elm Road Woods, Ryerson Conservation Area, and Wright Woods flatwoods, and neighboring mesic habitats that serve as key amphibian breeding and overwintering sites. Additional understory thinning and planting will be ongoing in MacArthur Woods and Grainger Woods. The planned work for the non-federal match includes 20 acres of understory removal and light thinning in the flatwoods ecosystem of Wright Woods in 2016, at \$1600/acre, 8.5 acres of hand clearing and understory removal in the flatwoods of Elm Road Woods in 2017 at \$2800/acre, and 8.5 acres of understory removal in mesic woodlands of Ryerson Conservation Area in 2018 at \$2800/acre. The non-federal match for restoration is broken down by project year (Table 2).

Table 2. Breakdown of LCFPD in-kind restoration work for the non-federal match.

Year	LCFPD Match	Restoration Phase
2016	\$32,000.00	20 acres of Wright Woods-Understory Removal
2017	\$23,800.00	8.5 acres of Elm Road Woods-Understory removal
2018	\$23,800.00	8.5 acres of Ryerson Woods-Understory removal
Total	\$79,600.00	

## **10. Multipurpose Projects**

n/a

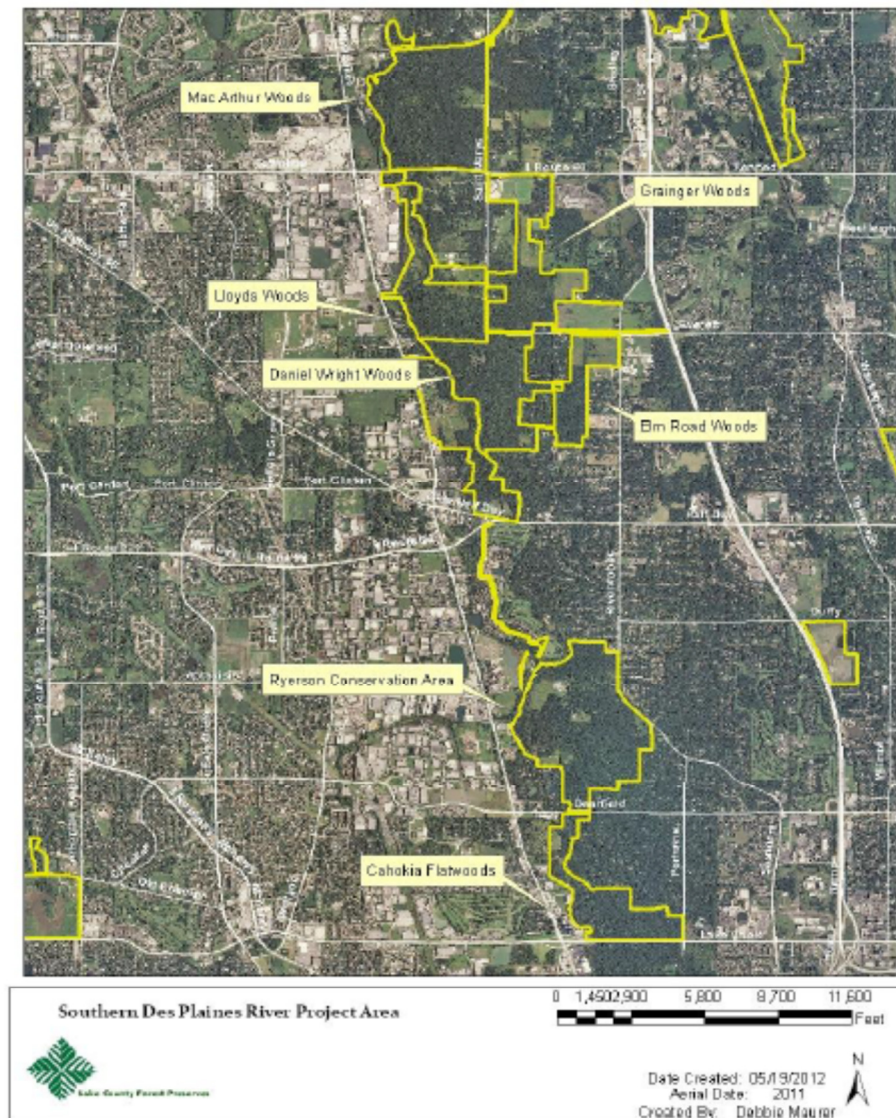
## **11. Relationship with other grants**

Grant # FW 98.18 for wooded wetland restoration and ecological monitoring of MacArthur Woods Forest Preserve (Final Report in 2003).

## **13. Timeline**

## SDPRP WOODLAND RESTORATION PLAN

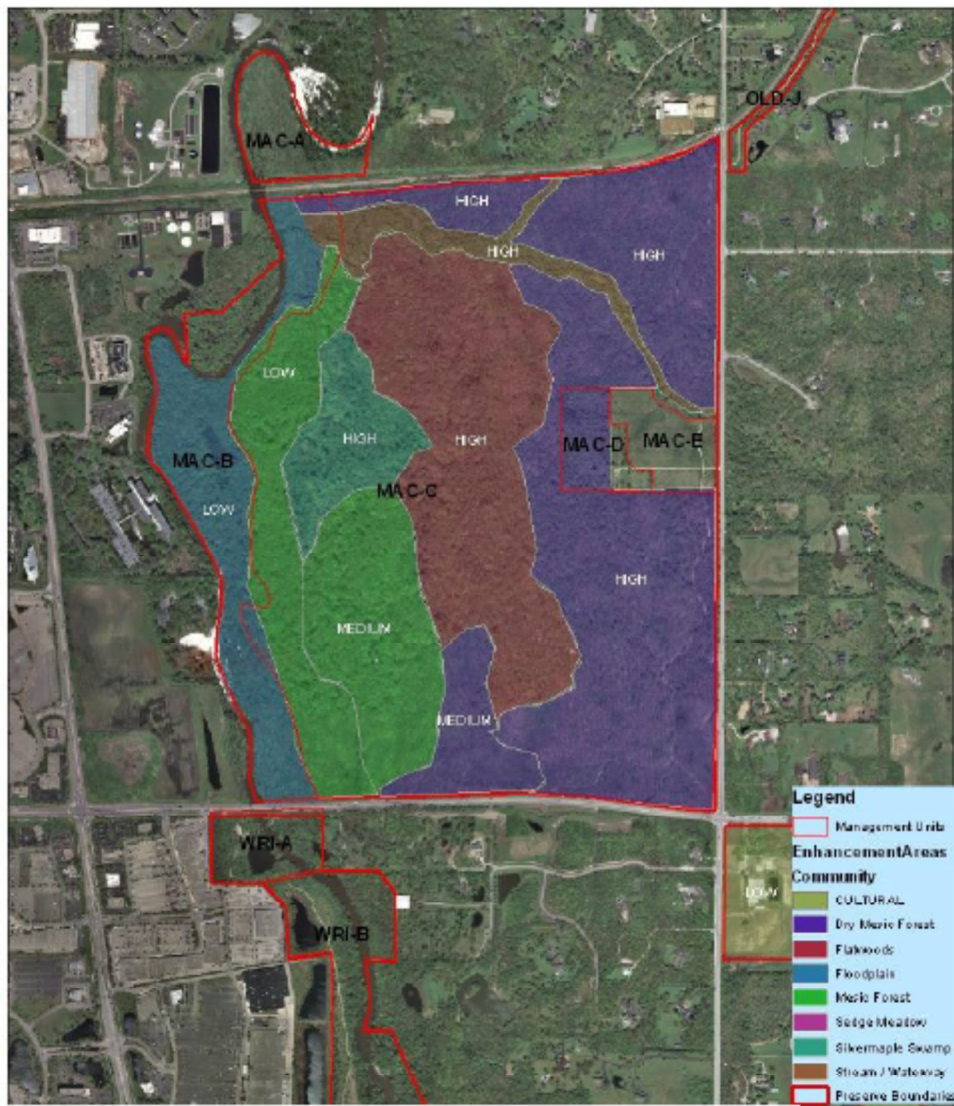
**Figure 1.** SDPRP Project Area Location





## SDPRP PHASE II RESTORATION

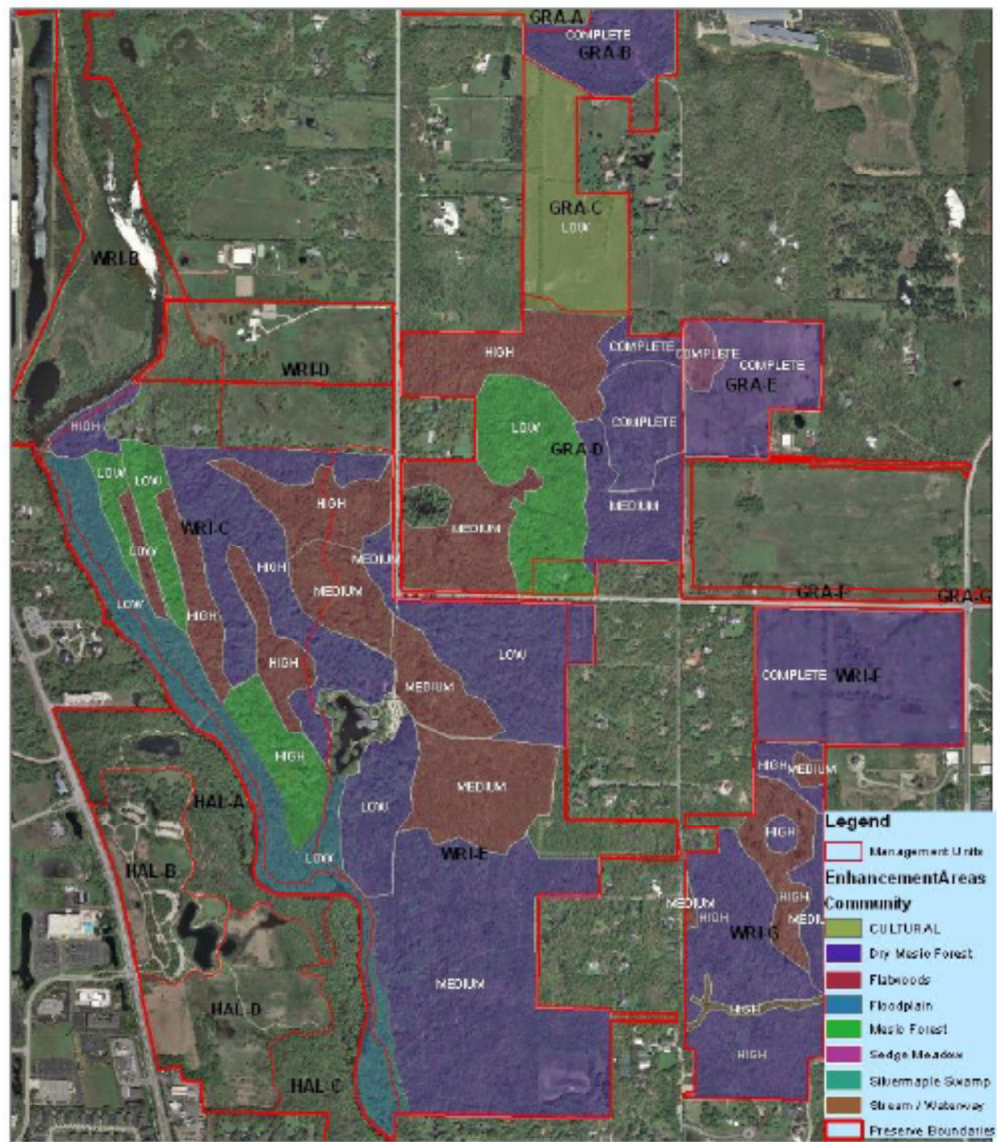
### Prioritized Project Areas



Project Area Priorities by Community Types in MacArthur Woods Forest Preserve



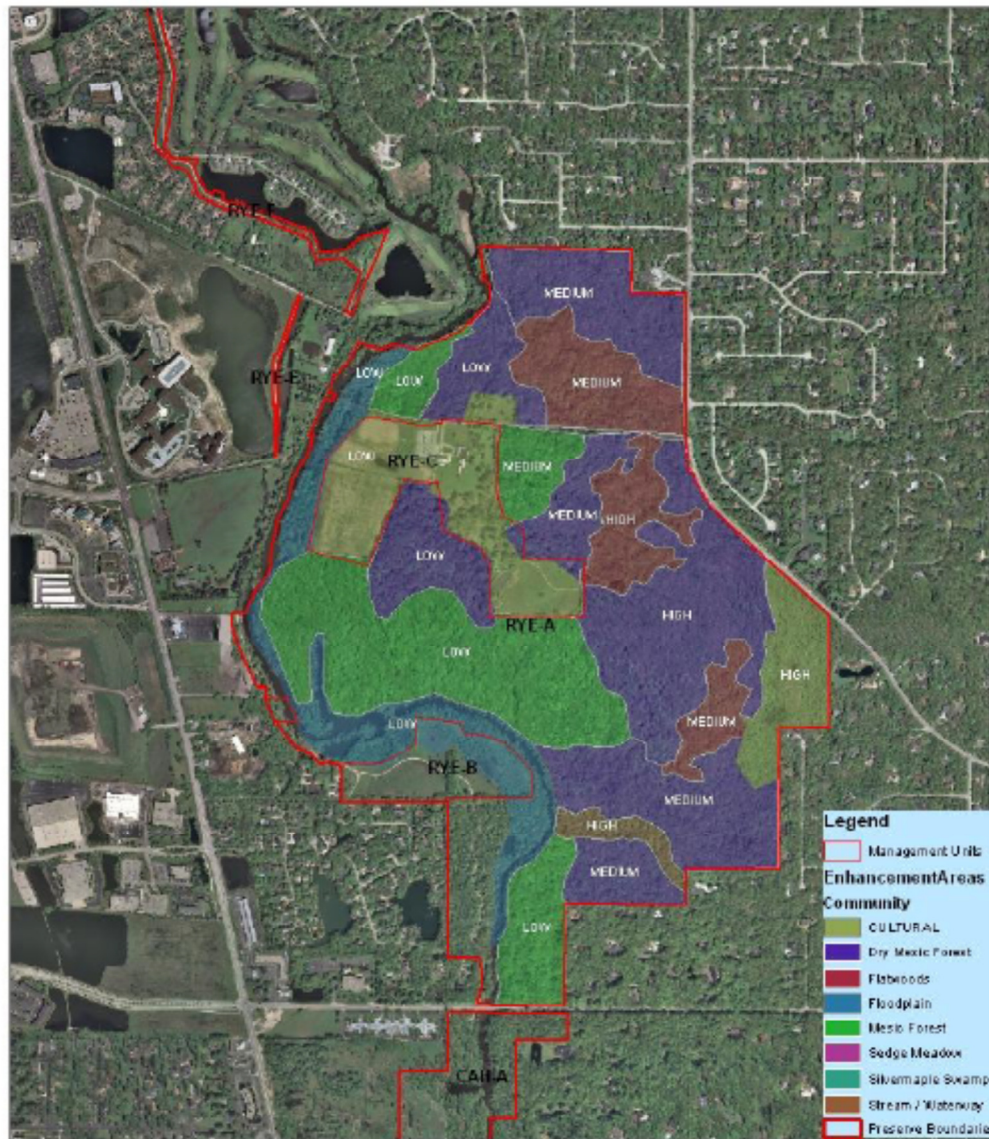
SDPRP PHASE II RESTORATION



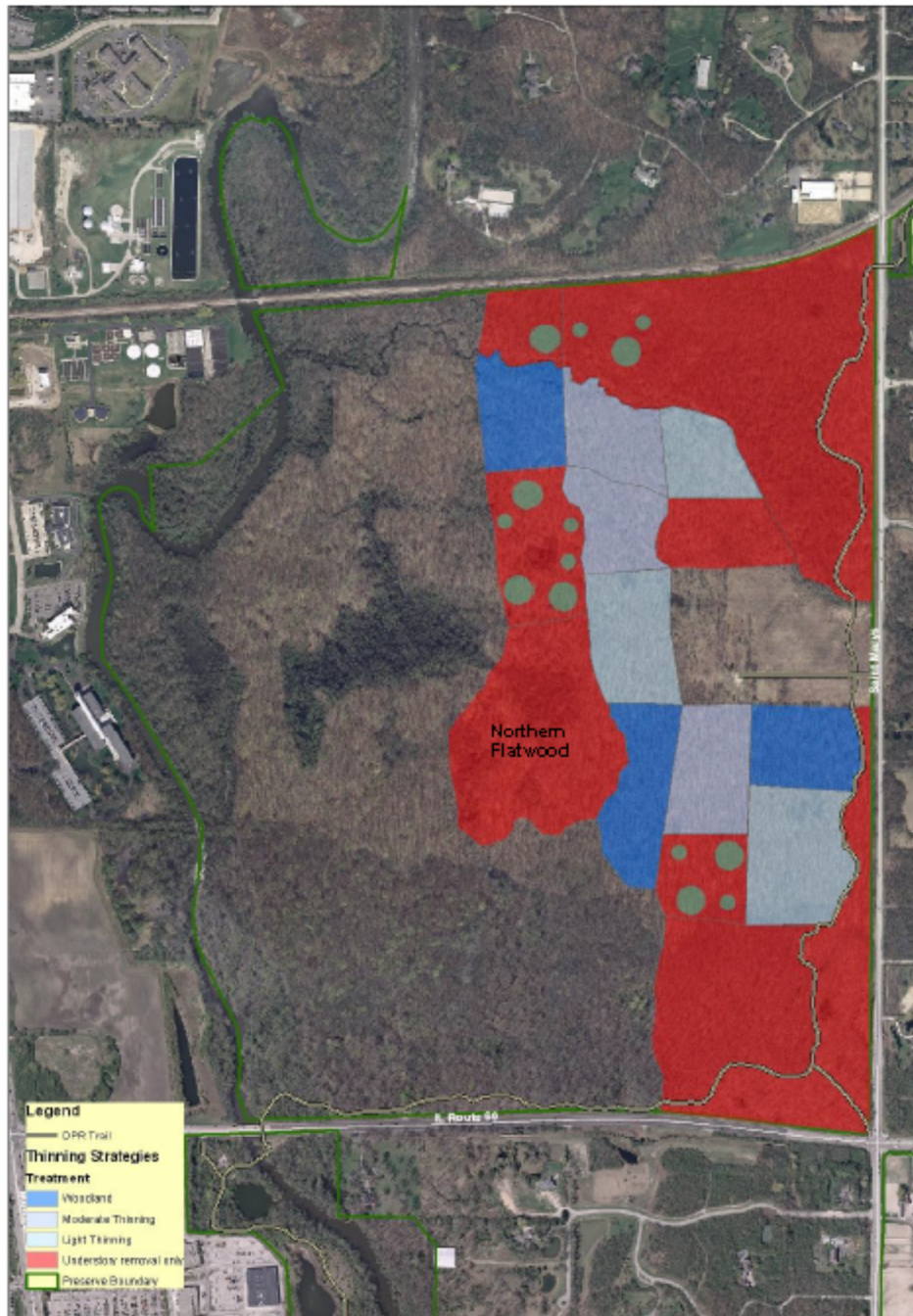
Project Area Priorities by Community Types in Wright Woods, Grainger Woods and Lloyds Woods Forest Preserves



## SDPRP PHASE II RESTORATION



## SDPRP PHASE II RESTORATION

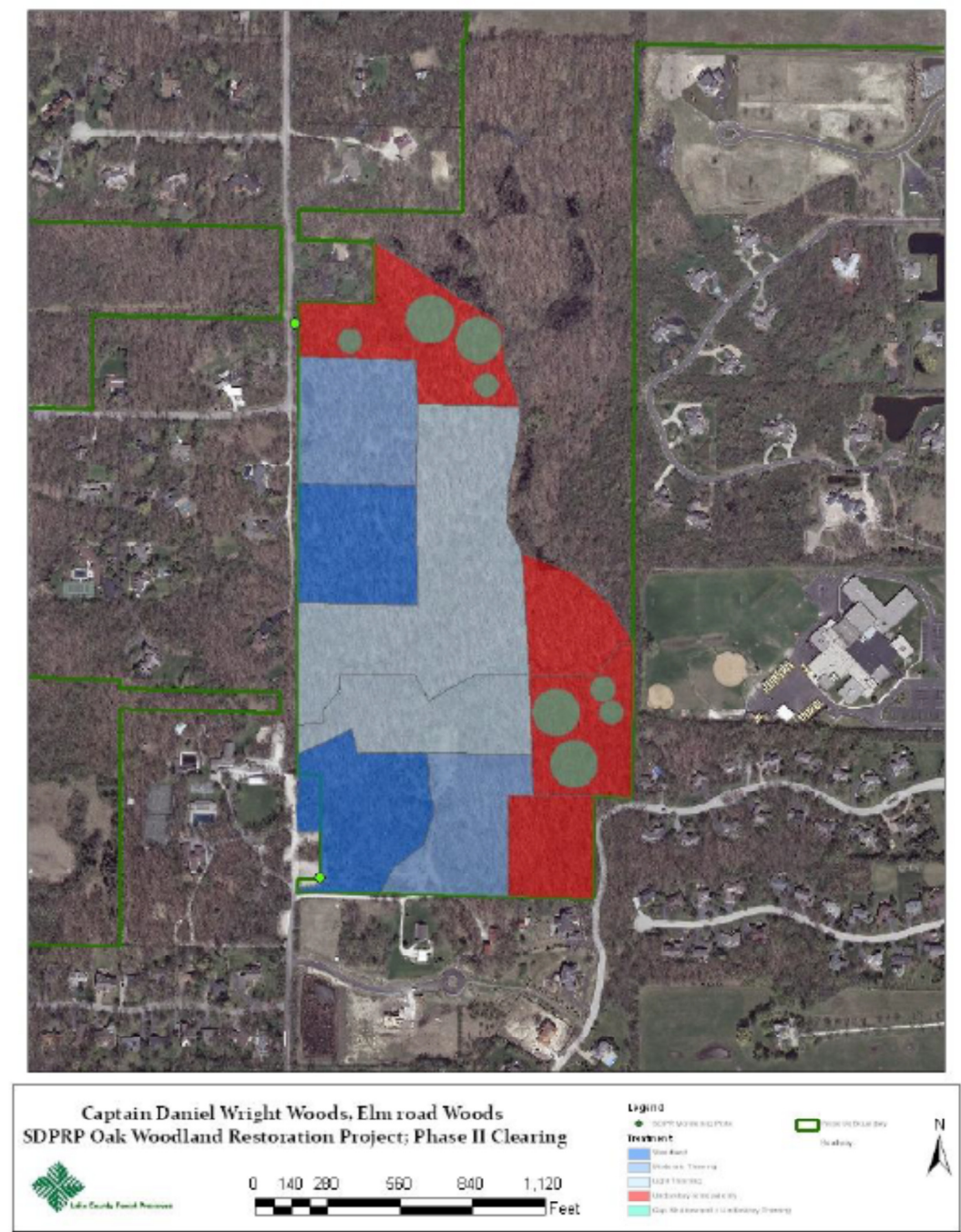


Woodland Habitat Restoration Plan  
MacArthur Woods

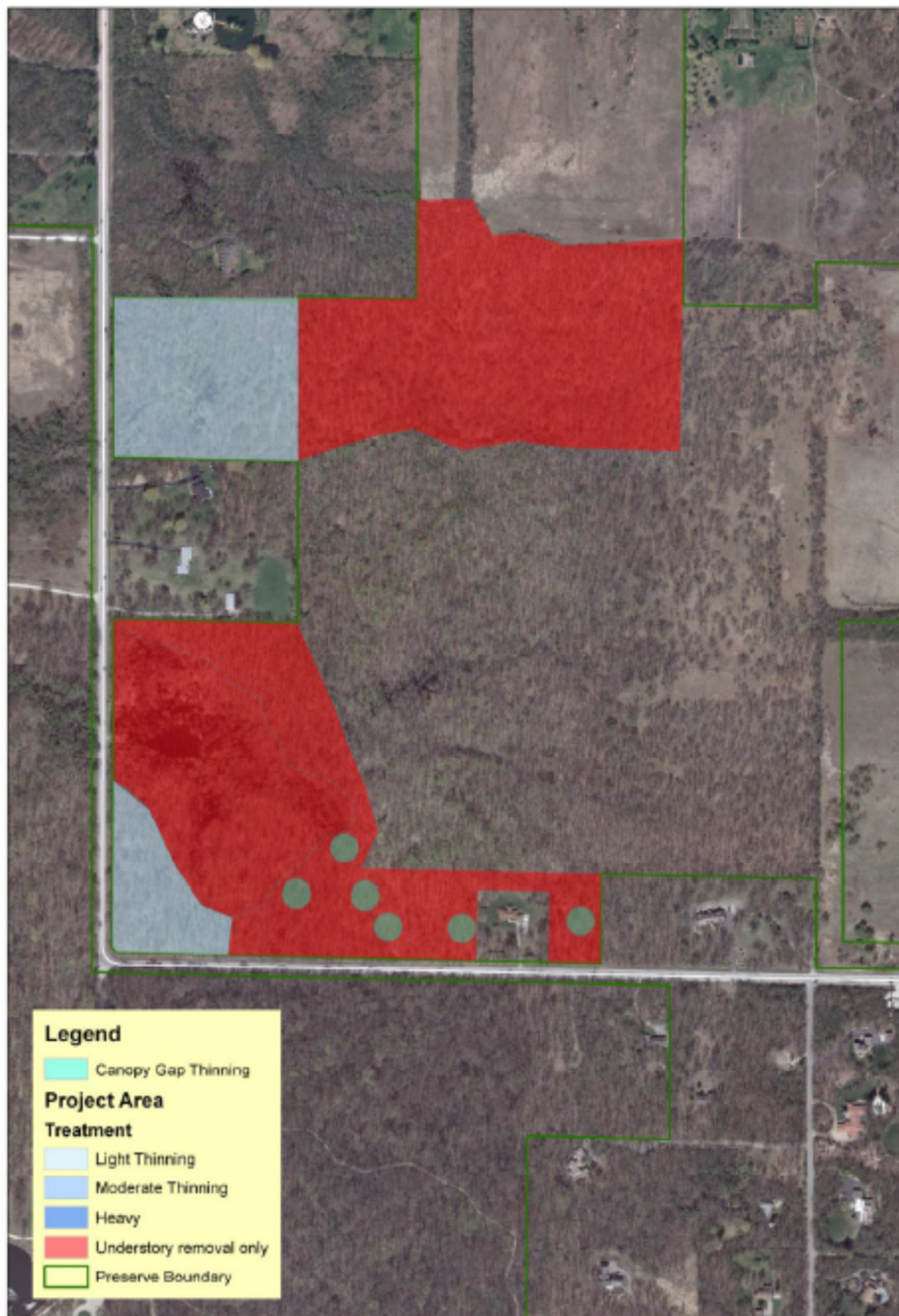




SDPRP PHASE II RESTORATION



## SDPRP PHASE II RESTORATION

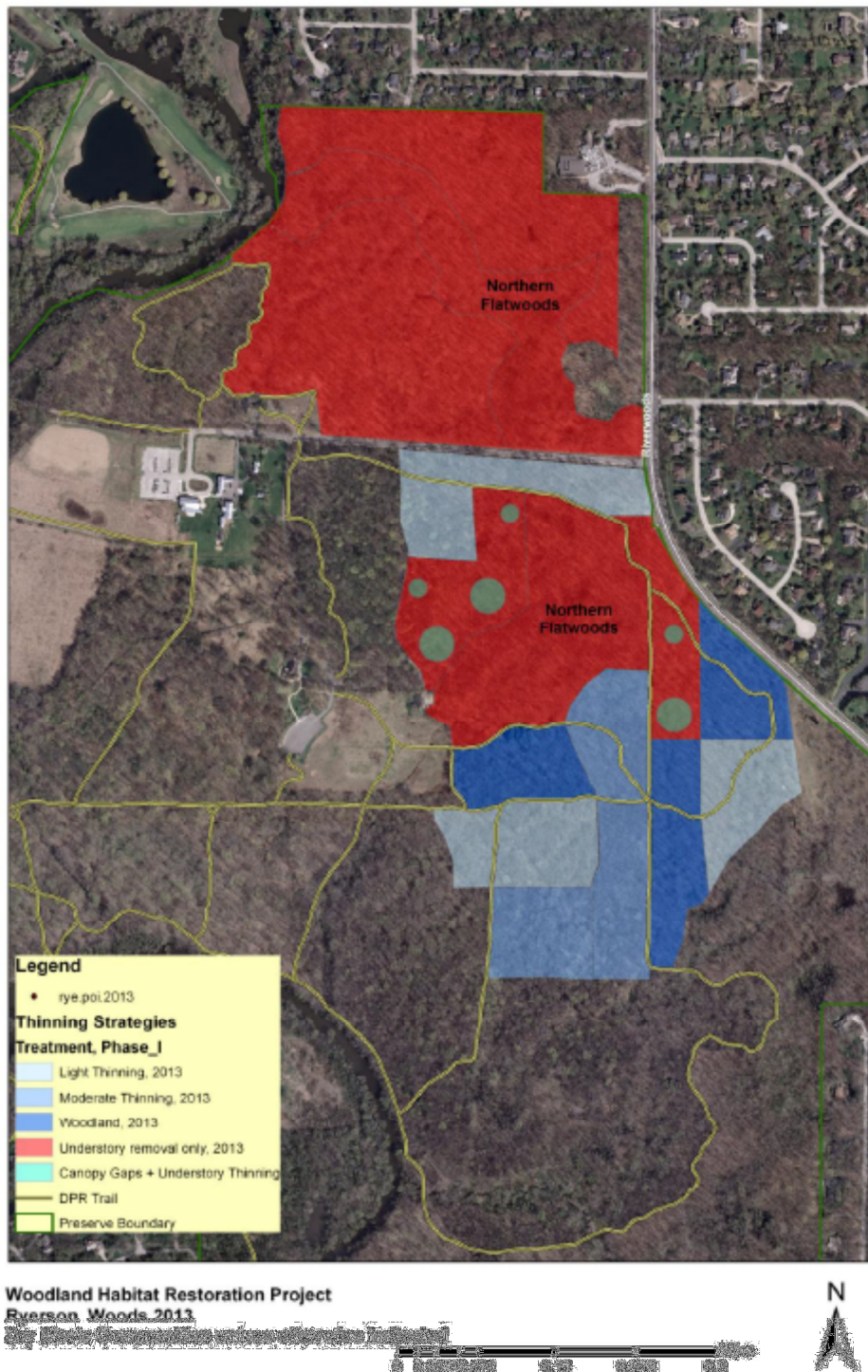


Woodland Habitat Restoration Project  
Greener Monitoring Plots





## SDPRP PHASE II RESTORATION



(i) General:

The project statement describes a need consistent with the State Wildlife Grants (SWG) program; states a purpose and sets objectives, both of which are based on the need; uses a planned approach, appropriate procedures, accepted principles of research, and is cost effective.

**(ii) Compliance:**

The IDNR will use its CERP (Comprehensive Environmental Review Process) as a tool to aid the Department in meeting NEPA compliance for the project outlined under this grant proposal. It is the Department's policy to require CERP applications for all land disturbing activities unless those activities are covered by CERP exemptions.

All planned activities will also be in compliance with the Endangered Species Act. All determinations and documentation will be in accordance with the current established U.S. Fish and Wildlife Service protocols for section 7.

All planned activities will be in compliance with the National Historic Preservation Act and the Council on Historic Preservation Act. All determinations and documentation will be in accordance with the terms of the Programmatic Agreement, as amended, effective September 23, 2002.

The activities supported by this grant for this project statement do not involve any other federal compliance issue.

**(iii) Literature Cited:**

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## Compliance

### **Appendix A. Benefitted Species of Greatest Conservation Concern and State-Listed Plants**

Wood Frog (SGCN)

Blue-Spotted Salamander (SGCN)

Downy Solomon's Seal (SE)

Grove Bluegrass (SE)

Hattorianus Bulrush (SE)

Heart-Leaved Plantain (SE)

Ill-Scented Trillium (SE)

Mountain Blue-Eyed Grass (SE)

Purple Fringed Orchid (SE)

Tuckermann's Sedge (SE)

Brome Sedge (ST)

Dog Violet (ST)

Downy Willow Herb (ST)

Dwarf Raspberry (ST)

Forked Aster (ST)

Marsh Speedwell (ST)

Pale Vetchling (ST)

Pretty Sedge (ST)

Swollen Sedge (ST)

Tubercled Orchid (ST)